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A PRIMITIVE COCCID CHROMOSOME CYCLE IN PUTO SP.

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Introduction

Every coccid thus far studied presents such striking peculiarities in its meiosis that we are confronted with the paradox of regarding a simple and orthodox maturation process as of especial interest. *Puto* sp. of the family Pseudococcidae reveals a primitive chromosome cycle possibly archetypal for coccids. Only in the llaveiine tribe of the family Margarodidae have partially comparable conditions been encountered. Thus in *Llaveia bouvari* we find as probably primitive traits a sex ratio which approaches equality, no trace of parthenogenesis nor of hermaphroditism, and an XX - XO sex chromosome mechanism. But even in this relatively generalized species a highly specialized achromatic figure has been evolved in male meiosis, and asynapsis of one pair of autosomes is already established as a constant and normal feature in a certain percentage of the spermatocytes. Moreover, in Llaveia the secondary pairing of homologous chromosomes just prior to the second meiotic division provides a mechanism which ensures segregation without previous synapsis—an essential preliminary step to the successful operation of the completely asynaptic habit as encountered in the related genus Protortonia. Puto, while it shares with Llaveia the primitive traits listed above, shows none of the specializations just enumerated. A survey of its cytology discloses a primitive, typically hemipteran pattern and further permits the recognition of certain phenomena as basic coccid characteristics independent of the specialized modifications encountered in the different groups.

Material and Methods

Specimens of this coccid have been deposited with Dr. Harold Morrison of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C., to whom grateful acknowledgment is made for his assistance. Dr. Morrison reports that the genus Puto is in so confused a state taxonomically as to preclude a specific identification at the present time.

The material was collected near the village of Tequisistlan, Oaxaca, Mexico, in November 1933. All instars were represented at this time, as again in more sporadic infestations found near Tehuantepec, Oaxaca, in December, 1938. The favorite host plant was the stinging-haired *Jatropha* known locally as the "mala mujer."

Male nymphs of the third and fourth instars and adult females with eggs and embryos were dissected in Allen's Bouin. This fixative gave good results in embryonic and late meiotic stages but proved unsuitable for early meiosis. Male material was sectioned at four and female at six micra, and stained in Iron Hematoxylin.

Chromosome Complement

The chromosomes of the female Puto are 14 in number and comprise 7 pairs differing slightly in length (Fig. 1). The male diploid set numbers 13, of which the next to shortest element is the unpaired sex chromosome (Fig. 2).

Somatic Mitosis

Somatic mitosis conforms to the hemipteran type. Its most characteristic features derive from the possession by the chromosomes of a diffuse, in contrast to a localized, kinetochore. Thus the whole body of the chromosome orients at metaphase, chromosomal fibers form from the poleward surface of each chromatid along its entire length, and anaphasic disjunction is parallel (Figs. 3, 4, and 5). In Puto the chromosomal fibers converge to division centers in which a minute centriole may often be discerned. Neither astral rays nor continuous fibers are present. The association between the constituent chromatids of the chromosome is closer throughout the mitotic cycle than in most coccids. (This effect is enhanced in the present material by the stain used.) Thus in the metaphase chromosome the two daughter chromatids only are usually distinguishable, although in an occasional end view a four-parted structure is suggested (Fig. 4). Anaphasic disjunction is parallel for about one third of the inter-center distance (Fig. 5); in late anaphase, as in most coccids, each chromosome curves toward the division center (Fig. 6).

Female Meiosis

The ovary of the young female conforms in structure to the usual coccid type. There is no trace of hermaphroditism in any instar. Meiosis is completely normal throughout its course. Seven normal bivalents are formed, and invariably two polar bodies are successively given off. This has been confirmed in many eggs, from several different females. Fusion of male and female pronuclei, while both polar bodies or their derivatives are still recognizable peripherally, has been observed in several eggs. Furthermore no haploid embryos have been found among some hundred checked. Thus, although no final conclusion is justified without the confirmation of breeding experiments, all the cytological evidence indicates the absence of parthenogenesis of either diploid or haploid type.

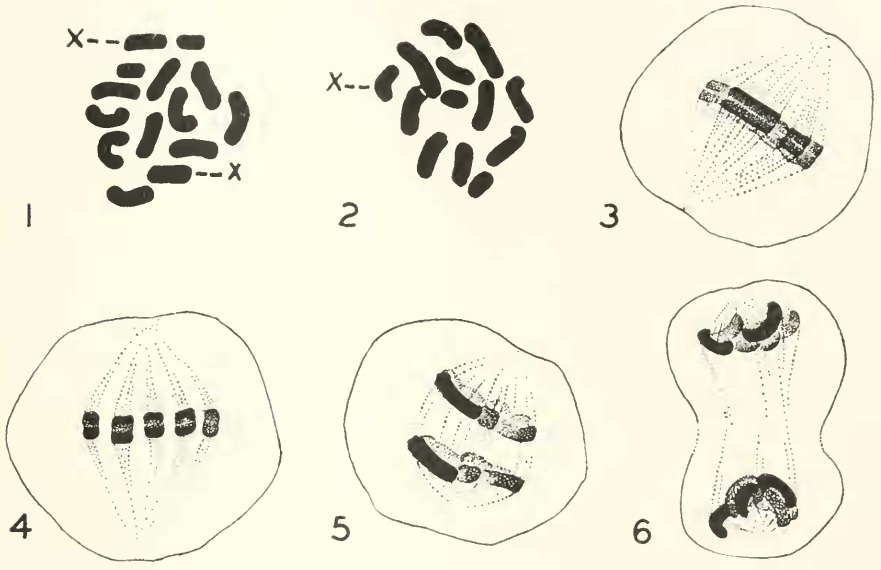
Male Meiosis

a. Prophases; structure and orientation of bivalents.

From diakinesis on, the major features of male meiosis can be followed with adequate clarity. Earlier stages fix too poorly for a detailed analysis but appear to be entirely normal. There is no evidence of any anomalous behavior, such as

vesicle formation, or a difference in rate of condensation between haploid sets of chromosomes, such as is associated with the variant degrees of asynapsis encountered in other coccids. A normal synapsis may safely be assumed. This is confirmed by the diakinetic bivalents. They are invariably six in number; no univalents other than the sex chromosome are present.

At diakinesis the autosomal bivalents and the sex chromosome are found peripherally distributed, closely underlying the nuclear membrane. The course of the constituent chromatids cannot be followed throughout the bivalents but open



FIGURES 1–6. Somatic mitosis. (All drawings made with camera lucida at table level with Zeiss 2 mm., 1.3 n.a. obj. and 20 × oc.; enlarged with pantograph; magnification as reproduced 2700 ×.)

FIGURE 1. Polar aspect of metaphase, female.

FIGURE 2. Same, male.

FIGURE 3. Lateral aspect of metaphase; entire chromosome oriented, chromosomal fibers from entire length of chromosome.

FIGURE 4. Same—one focal level only drawn—showing ends of a group of chromosomes.

FIGURE 5. Early anaphase—disjunction parallel.

FIGURE 6. Late anaphase—chromosomes curve toward centers.

cross configurations (center, Fig. 7) suggest the resolution of a chiasma by rotation of the arms. Bivalent C of figure 8 would similarly be interpreted as a later stage in the same process. But the question of chiasmata aside, it is evident that in the marginally placed bivalents of figure 7 and in A and B of figure 8, the homologous chromosomes of each bivalent are assuming an end to end juxtaposition. In the interpretation of these bivalents it must be remembered that no localized kinetochore is present in these chromosomes. The median knots in bivalents such as A and B in figure 8 thus represent chromosome ends and not, as might be assumed on superficial scrutiny, kinetochores. Similarly, the large central

aperture of the bivalent separates originally sister chromatids in the vertical, and homologous chromosomes in the horizontal arms.

b. Metaphase I.

Shortening and thickening of the chromosomes proceed rapidly and with no change in the position of the homologues in relation to each other. By metaphase each bivalent is a compact, superficially four parted body; but extreme as is the condensation undergone, a polar view of an early metaphase plate (Fig. 9) still gives evidence of the end to end alignment of homologues in some of the bivalents. In the metaphase orientation, as expected under the influence of the diffuse kinetochore, the long axis of each chromosome lies at right angles to the spindle axis. The constriction visible in each bivalent from the polar view is therefore the primary split—in this case the point of contact between the ends of the two homologous chromosomes. The constriction visible from the lateral aspect (Fig 10), which becomes the plane of separation in the ensuing division, is accordingly the secondary split. The first division, patently equational for the X chromosome, is thus, disregarding crossing-over, basically equational in character for the autosomal bivalents also. In structure and orientation for the first division, therefore, the bivalents of Puto conform to the coccid and aphid type, as analysed by Ris (1942).

c. First meiotic division.

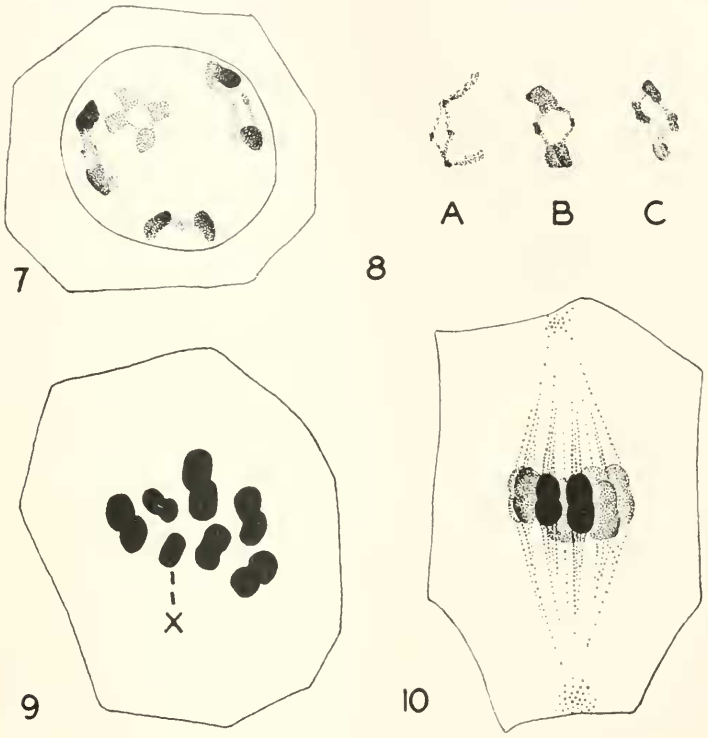
Chromosomal fibers form from the entire poleward surface of each chromatid and converge toward the division center. The four wefts of fibers thus produced in relation with each bivalent are visible in the obliquely viewed, leftmost bivalent of figure 10. No centrioles are visible and the chromosomal fibers tend to fade out distally, but the pole is nevertheless well demarcated and the spindle as a whole is of normal form. As in somatic mitosis no continuous fibers nor astral rays are present. Anaphasic disjunction is normal and regular with no trace of differential rates among the bivalents or their constituent elements. Delicate interzonal connectives form between the separating chromosomal elements, but the small size precludes an analysis of their structure.

d. Interkinesis: separation and secondary pairing of chromatids.

Already in the telophase of the first division the two chromatids derived from each metaphase bivalent begin to separate (Fig. 11). In the ensuing interkinetic interval this movement is continued until frequently the separation is complete and the full diploid number of chromatids may be counted as in figure 12. This separation is not interpretable as an extreme expression of that "repulsion" between chromatids characteristic of most organisms immediately prior to the second meiotic division. The chromatids here involved are not originally sister strands held together by a joint kinetochore region, but represent, again disregarding crossing-over, equational halves of the two homologous chromosomes of the metaphase bivalent. Their separation thus indicates simply the lapse of the terminal attraction or association which held the homologues together after the terminalization of any chiasmata which may have been present—an association ordinarily broken at first anaphase.

Little or no unravelling of the chromatids has thus far occurred. They remain throughout interkinesis as compact centers with only a slight irregularity of outline

(Figs. 12 and 13). The nuclear membrane now reforms, and it is of interest that therewith the chromatids assume once more, as previously in diakinesis and later in the spermatid nucleus, a peripheral distribution underlying the membrane. Simultaneously with this orientation the chromatids begin to reassociate in pairs (Fig. 13). Size differences show this pairing to be between homologous chromatids. Although the long axis of these compact chromatids cannot now be deter-



FIGURES 7–10. Diakinesis and Metaphase I in male.

FIGURE 7. Diakinesis—(only four bivalents drawn); three bivalents show homologues assuming end to end position, one open cross.

FIGURE 8. Diakinetic bivalents; *A*—early assumption of end to end position of homologues; *B* and *C*—stages in opening of cross configuration.

FIGURE 9. First meiotic metaphase, polar view; six bivalents and univalent ×; constriction in bivalents is primary split.

FIGURE 10. Same, lateral view; constriction in plane of separation is secondary split.

mined with accuracy, there is little doubt that the realignment results in a side by side lengthwise, association. This assumption is supported by the close parallellism obtaining between the realignment here and in the corresponding chromatids of Nautococcus (Hughes-Schrader, 1942) in which the long axis is persistently recognizable. Moreover, as the newly formed dyads orient for the second metaphase the plane of contact between the homologous chromatids comes to lie at right angles to the spindle axis and forms the plane of separation for the

second division. Chromosomal fibers then form from the entire poleward surface of each chromatid further identifying this as the long axis. The second division is thus reductional for all non-crossover regions.

It should be emphasized that the seriation of the interkinetic stages just described can be positively established. Cell and nuclear size are in series with those of first anaphase and telophase on the one side, and second metaphase on the other. Moreover, in the first telophase alone is a heavily staining midbody developed in the interzonal connectives (Figs. 11 and 12). This midbody is retained, with decreasing sharpness of staining reaction, in the interkinetic cells and thus confirms their identification.

c. Second meiotic division.

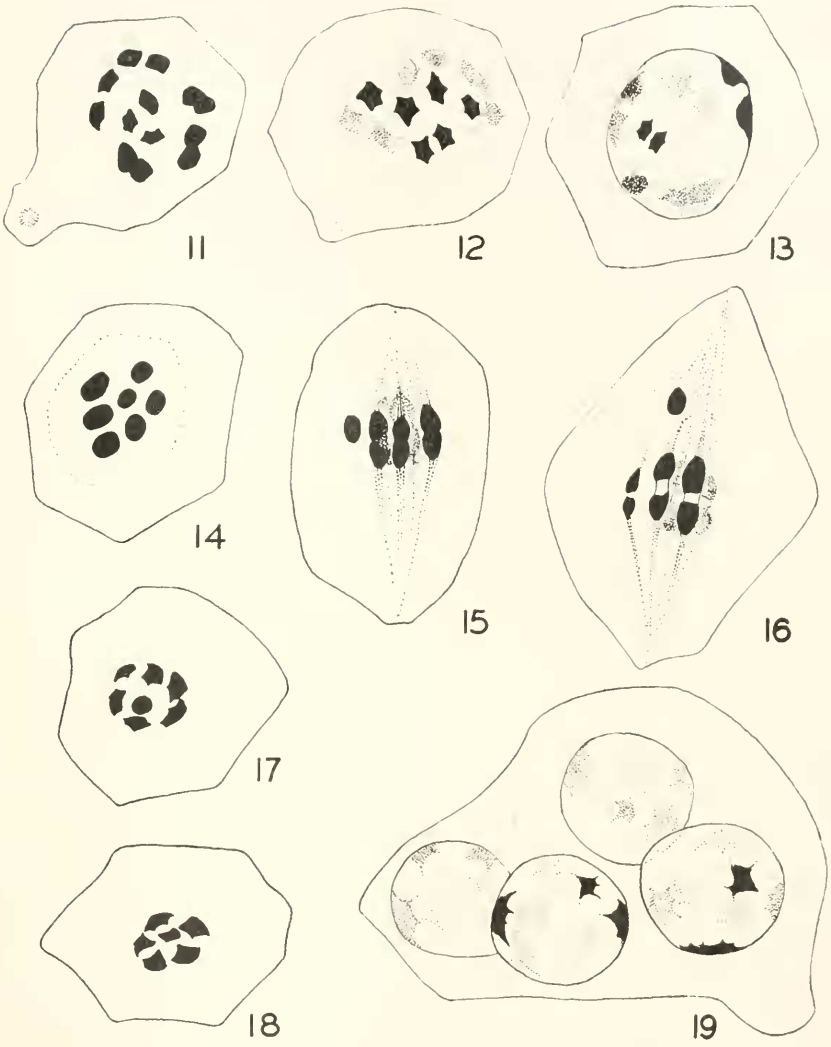
The spindle for the second division resembles that of the first, but with more sharply delimited and acuminate ends. Again chromosomal fibers alone are formed, neither continuous fibers nor astral rays being present (Figs. 15 and 16). The sex chromosome comes to lie either on the edge of the equatorial plate, or more frequently nearer to one pole, but always close to the spindle. It shows no tendency toward division, produces no chromosomal fibers, and passes usually in advance of the autosomes undivided to one pole. Second telophases show the expected two categories of spermatid nuclei—those with six autosomes and the X (Fig. 17) and those with six autosomes only (Fig. 18).

f. Quadrinucleate spermatids.

With the formation of a membrane around the spermatid nucleus the chromosomes again assume a peripheral position under it (Fig. 19). A cytoplasmic fusion of the spermatid cells in groups of four now takes place. Each resultant quadrinucleate spermatid contains, invariably, two of the six-chromosome and two of the seven-chromosome nuclei (Fig. 19). It follows that the four nuclei involved thus almost certainly represent the products of a single primary spermatocyte. The short centrally directed stalk often visible in the body of the quadrinucleate cell further suggests that the meiotic divisions may not have been quite complete cytoplasmically. If the products of the two divisions retain a connection through a common stalk a limiting factor in the fusion would be provided. Such a mechanism, it will be recalled, has been demonstrated in other coccids (F. Schrader, 1931, Hughes-Schrader, 1931). Later stages in sperm formation show the progressive development of all four components of the quadrinucleate spermatid. There is no evidence of any degeneration or loss of nuclei and apparently four normal sperm are formed from each quadrinucleate spermatid.

Comment

Cytologically *Puto* sp. stands out as a persistently primitive type among coccids thus far studied. This is evident in the absence of hermaproditism and of parthenogenesis and in the retention by both male and female of a normal meiosis. Its relatively generalized chromosome cycle is most nearly approached by the more primitive species of the Ilaveiine tribe of the family Margarodidae. Taxonomically the Margarodidae and the Ortheziidae constitute the most primitive subdivision of existing coccids; they are set off from all other families by such primitive traits



FIGURES 11–19. Interkinesis and second meiotic division in male.

FIGURE 11. Late telophase 1 with separation of chromatids underway; spindle rest of first division at lower left.

FIGURE 12. Early interkinesis; complete separation of chromatids.

FIGURE 13. Reassociation of chromatids in pairs; spindle rest usually present at this stage not included in section.

FIGURE 14. Polar view of second metaphase.

FIGURE 15. Lateral view of same; × chromosome close to spindle at equator.

FIGURE 16. Early second anaphase; × chromosome near spindle, off equator.

FIGURE 17. Second telophase, with 6 autosomes and × chromosome.

FIGURE 18. Same, with 6 autosomes only.

FIGURE 19. Quadrinucleate spermatid, with two nuclei showing 7 and two 6 chromosomal masses.

as the retention (with a few specialized exceptions) of abdominal spiracles in all stages and well developed compound eyes in the adult males. Their closest relatives among other coccids are to be found in the Pseudococcidae—of which Puto constitutes the probably most primitive genus—linking the pseudococcid and ortheziid stems (Morrison, 1928, and personal communication).

The persistence in the family Pseudococcidae of so primitive a type of chromosome cycle as that of Puto has especial interest in view of the highly specialized male meiosis of the other pseudococcids thus far investigated. These comprise several species of Pseudococcus (Schrader, 1921, 1923a and b), and *Phenacoccus acericola* (Hughes-Schrader, 1935). These are jointly characterized by a persistent heteropycnosis of one haploid set of chromosomes in the male, by segregation without synapsis, and by the degeneration of the spermatid nuclei derived from the heteropycnotic complement. Similar conditions are encountered in *Gossyparia spuria* of the family Kermidae (Schrader, 1929) and in *Lecanium hesperidum* and *L. hemisphaericum* of the Coccidae (Thomsen, 1927, Suomalainen, 1940). While Puto throws no light on the origin of these specializations, the existence of the XX - XO sex chromosome mechanism in a primitive pseudococcid is highly significant, indicating that the male is primarily the heterogametic sex in this group. Its presence alike in Puto and the primitive Ilaveiines may well mean that it also represents the primitive condition for coccids as a whole. Its loss, and the substitution of alternative mechanisms—(haplo-diploidy in the Iceryini and the as yet unsolved sex determining mechanism of Pseudococcus, Phenacoccus, Gossyparia, and Lecanium in which both sexes originate from eggs fertilized by one class of sperm only)—have occurred in all other forms thus far investigated. Homogamety of the female relative to sex is indicated by the fact that in all cases the eggs of diploid-parthenogenetic females and of self-fertilized hermaphrodites (basically female in constitution) give rise exclusively to females.

The curious cytoplasmic fusion of spermatids in groups of four appears to be of very early origin in the coccid stem for it is found in every species thus far studied. Even the haploid males of the Iceryini with only one meiotic division retain the habit, producing binucleate spermatids. Multinucleate spermatids have been described in certain spiders by Wagner (1896). He reports variation within the individual and among species; the binucleate and quadrinucleate condition is frequent and higher multiples are occasionally encountered. Later authors have not dealt with the problem in detail but incidental observations (Wallace 1905, Bösenberg, 1905, and Chickering and Hard, 1935) indicate that cell bridges containing spindle remnants frequently persist between spermatids. Incomplete cytoplasmic division and multinucleate cells probably form the basis for certain of Warren's (1928, 1931) claims of amitosis in spider spermatogenesis. In the coccids no variation among species nor within the species or the individual has been observed. The fusion is always limited to the derivatives of each primary spermatocyte. The limiting factor appears to be the persistence, in the radially arranged cells of each cyst, of a centrally directed stalk from each spermatocyte—a stalk never completely severed during the meiotic cell divisions.

A significant feature of male meiosis in Puto is the complete separation and subsequent realignment of the chromatids during interkinesis. As already pointed out, this separation breaks the terminal association between homologous chromo-

somes which persists throughout the first division, and the realignment side by side ensures segregation at the second division. This type of meiosis, with its characteristic and essential orientation of the bivalents at first metaphase, is found in all the more primitive of the llaveiine coccids (Llaveia, Llaveiella, and Nautococcus—Hughes-Schrader, 1931, 1940, 1942). Although the meiotic figures of the female Puto are too small for critical analysis, it is significant that in the females of *Pseudococcus citri* (Schrader, 1923a) and *Lecanium hesperidum* (Thomsen, 1927), which in contrast to their highly specialized males retain an otherwise orthodox meiosis, the same separation and realignment of chromatids for the second division take place. Its occurrence in the unspecialized Puto male further confirms the conclusion that this type of meiosis is a primitive character for the coccids as a whole. Ris (1942), who first pointed out the significance of these phenomena, presents convincing evidence that the same type of meiosis obtains in aphids also, and must thus have differentiated after the Sternorhyncha had separated from the auchenorhynchous Homoptera. Incidentally it is of interest that the secondary pairing involved in this type of meiosis may well have played a role in the evolution of asynapsis among the llaveiine coccids. With the renewed operation of the pairing force just prior to the second division, asynaptic chromosomes which have divided separately and equationally during the first division, are brought together briefly at second metaphase and undergo a normal segregation. Thus in the llaveiine coccids asynapsis has been free to evolve without its usual sequelae of meiotic irregularities. Secondary pairing, while completely independent in its origin, here incidentally operates as a mechanism stabilizing asynapsis.

Summary

A primitive chromosomal cycle possibly archetypal for coccids is reported for *Puto* sp. of the family Pseudococcidae. There is no hermaphroditism nor any cytological evidence for parthenogenesis. The diploid chromosome number is 14 in the female, 13 in the male. Somatic mitosis is of the type characteristic for chromosomes with diffuse kinetochore. Meiosis is regular in both sexes. In the male it can be demonstrated to adhere to the coccid-aphid type, with: (*a*) the first division equational for non-crossover regions; (*b*) separation of chromatids and their secondary pairing during interkinesis, and (*c*) segregation of non-crossover regions in the second division. An XX-female, XO-male sex determining mechanism is present. Quadrinucleate spermatids are formed. This is the only coccid thus far reported with a simple and orthodox meiosis in both sexes.

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